Invertebrate Reproduction & Development

ISSN: 0792-4259 (Print) 2157-0272 (Online) Journal homepage: https://www.tandfonline.com/loi/tinv20

Histochemical study of the ovarian development of the blue land crab *Cardisoma guanhumi* (Crustacea: Gecarcinidae)

Renata A. Shinozaki-Mendes , José Roberto F. Silva , Leonardo P. de Sousa & Fábio H.V. Hazin

To cite this article: Renata A. Shinozaki-Mendes , José Roberto F. Silva , Leonardo P. de Sousa & Fábio H.V. Hazin (2012) Histochemical study of the ovarian development of the blue land crab *Cardisoma guanhumi* (Crustacea: Gecarcinidae), Invertebrate Reproduction & Development, 56:3, 191-199, DOI: 10.1080/07924259.2011.587277

To link to this article: https://doi.org/10.1080/07924259.2011.587277

Invertebrate Reproduction & Development
Vol. 56, No. 3, September 2012, 191–199

Taylor & Francis
Taylor & Francis Group

Histochemical study of the ovarian development of the blue land crab *Cardisoma guanhumi* (Crustacea: Gecarcinidae)

Renata A. Shinozaki-Mendes[a]*, José Roberto F. Silva[b], Leonardo P. de Souza[b] and Fábio H.V. Hazin[a]

[a]*Depto. Pesca e Aqüicultura, Universidade Federal Rural de Pernambuco, Av. Dom Manuel de Medeiros, s/n Dois Irmãos CEP 52171900, Recife/PE, Brazil;* [b]*Depto. Biologia, Universidade Federal do Ceará, Av. Mister Hull, s/n Pici – Bloco 909 CEP 60455760, Fortaleza/CE, Brazil*

(*Received 14 December 2010; final version received 12 April 2011*)

The reproductive tract of female *Cardisoma guanhumi* was macroscopically and microscopically described. The stages of sexual maturation were defined and related to gonad coloration and to the degree of development of the germ cells, including oogonia (diameter $14.17 \pm 0.97\,\mu m$), forming nests in the germ zone; pre-vitellogenic oocytes (PVO, $51.02 \pm 2.90\,\mu m$) of a basophilic nature, with one or two nucleoli; vitellogenic oocytes (VO, $110.93 \pm 7.04\,\mu m$) of acidophilic nature; mature oocytes (MO, $237.52 \pm 13.54\,\mu m$), revealing the chorion; and atretic oocytes (AO). Follicular cells were found close to germ cells. The following gonad development stages were identified: immature (translucent color, germ zone containing oogonia, and peripheral maturation zone with PVO); maturing (color ranging from yellow to dark brown; maturation zone with VO); mature (dark brown color; predominance of MO in maturation zone); spawning (dark brown or yellow color, containing oocytes in early atresia, stage observed in ovigerous females); and resting (translucent to yellow color, thick gonad wall, and residual AO).

Keywords: Brachyura; histology; reproductive biology; oocyte

Introduction

The reproductive cycle of *Cardisoma guanhumi* Latreille, 1825 is heavily dependent on season, beginning with the rainy season. Following copulation and internal fertilization, females carry their eggs (between 20,000 and 1,200,000) for approximately 2 weeks before releasing them into the ocean. Despite this adaptation to terrestrial life, *C. guanhumi* depends on the sea to release the eggs, in search of which it often crosses long distances (Burggren and McMahon 1988).

Decapod ovaries are paired organs located in the cephalothorax, which vary in shape, size, and color (e.g., Castiglioni et al. 2007). Although the macroscopic characteristics of the ovarian is an important element to characterize the stages of the ovarian development, for an adequate comprehension of the female reproductive cycle, therefore, it is necessary to describe the maturation of germ cells in the ovaries (Adiyodi and Subramonian 1983).

The development of the reproductive cycle in crustaceans includes the proliferation of cells in ovaries, growth toward maturation, ovulation, extrusion of eggs, and incubation until larval hatching (Sastry 1983). Histological descriptions of the female reproductive organ of brachyuran crustaceans have been published by Johnson (1980), Santos et al. (2009), Rjeibi et al. (2010), Islam et al. (2010), Diez and Lovrich (2010), and others.

The information on ovarian development of a given species is very important to adequately understand its population dynamics, from the aspects of reproduction, such as growth characteristics and differences among populations. Thus, this information is crucial for the development and implementation of management strategies that can ensure the long-term sustainability of the exploited stocks.

The aim of this study was thus to gain a better understanding of the oogenesis and ovarian maturation of female *C. guanhumi*, through macroscopic and microscopic descriptions of the reproductive system, including the development stages of the germ cells.

Materials and methods

The specimens were collected from the estuary of the Jaguaribe River (04°26′S to 04°32′S and 037°46′W to 037°48′W) in the eastern coast of the state of Ceara (Brazil) in the Cumbe mangrove area, approximately 2 miles from the mouth of the river. In the 12-month period between December 2006 and November 2007, 177 females between 4.34 and 7.93 cm in carapace width were collected, including ovigerous females.

*Corresponding author. Email: renataasm@gmail.com

ISSN 0792–4259 print/ISSN 2157–0272 online

http://dx.doi.org/10.1080/07924259.2011.587277
http://www.tandfonline.com

The specimens were caught with artisanal traps, of about 25 cm length and 10 cm diameter, deployed at night at the opening of the burrows and collected in the following morning. The individuals were immobilized by thermal shock at −10°C during 15 min (meaning that the crabs were not allowed to freeze) in a refrigerator prior to dissection. After that, the carapace was cut laterally in order to allow the removal of the reproductive tract.

The location of the reproductive tract in the cephalothorax was recorded prior to the removal. For the determination of ovarian color, the red, green, and blue (RGB) scale was used, as proposed by Robinson et al. (1995). In this scale, a given color is expressed as the result of the combination of these three colors, expressed in numbers.

Sections from the anterior, middle, and posterior regions of the gonads and the spermathecae were fixed in Davidson's solution (Shaw and Battle 1957) for 24 h and then kept in a 70% ethanol. Sections were submitted to be dehydrated in an increasing ethanol series, followed by clearing in xylol and impregnation and embedding in paraffin at 60°C. The paraffin blocks were then cut into 5-μm sections and stained using the following methods: Bromophenol Blue, Alcian/PAS (PAS, Periodic Acid Schiff) Blue, and Gomori's Trichrome stain (adapted by Pearse 1960; Tolosa et al. 2003). Slides were analyzed with an optical microscope, with objectives from 4 × to 100 × of magnification.

The germ cells were classified in distinct stages based on shape, nucleus–cytoplasm ratio, and reaction to stains. The diameters of germ cells were measured in the histological cross-sections. When present, the germ cells were also measured and classified into four distinct stages based on shape, nucleus–cytoplasm ratio, and reaction to stains. Only those cells that had clearly visible nucleus were measured for maximum diameter as well as nucleus diameter. The different phases of ovarian development were determined based on the proportion of cells in each stage. The microscopic and macroscopic characteristics were compared in order to better understand the reproductive biology of the species.

The nucleus and cell diameter of the different stages of germ cells during oogenesis were measured. Data were tested for normality (D'Agostino-Pearson) and homoscedasticity (Bartlett's test), and then, for oneway ANOVA ($p < 0.05$), followed by a Tukey's test (Zar 1984; Mendes 1999). The nucleus-to-cell ratio was calculated as percentage dividing the diameter of the nucleus to the cell.

Results

Anatomy

The female reproductive tract of *C. guanhumi* is formed by paired ovaries and spermathecae connecting to the gonopore. The ovaries are dorsal organs, arranged as two elongated lobes interconnected by a transversal fissure below the heart. The posterior portion of the ovaries has a lateral expansion in the shape of a sac, named spermatheca (Figure 1). The gonopores, which are visible to the naked eye, are located on the sternite at the level of the third pair of pereiopods. The volume, length, and color of the gonads vary in accordance with the degree of development of the germ cells.

Macroscopic description

The size of gonads ranging from quite small, with color varying from transluscent to yellow and occupying only the central portion of the cephalothorax (Figure 1(A) and (B)), to reaching maximum ovary diameter and length, occupying the entire empty space of the cephalothorax (Figure 1(C) and (D)), with color ranging from yellow, orange, light brown to dark brown.

Microscopic description

Description of oocytes and auxiliary components

The germ cells exhibited a constant increase in their mean diameter throughout the development process (Table 1). The oogonia were the smallest cells, followed by the pre-vitellogenic oocytes (PVO), and by vitellogenic oocyte (VO) and mature oocyte (MO) ($p < 0.05$). The diameter of the nuclei exhibited the same trend ($p < 0.05$), but with less variation.

Oogonia (OO). Diameter from 9 to 26 μm. The oogonia form clusters in the central region of the gonad (germ zone) (Figure 2(A) and (F)). The initial stage has a thick cytoplasm in relation to the visible nucleus (Figure 3(A)). Central chromatin, considerably condensed in the nucleus, stained strongly purple by Alcian/PAS and Gomori's Trichromic stain indicates a basophilic nature. In the advanced stage, the more

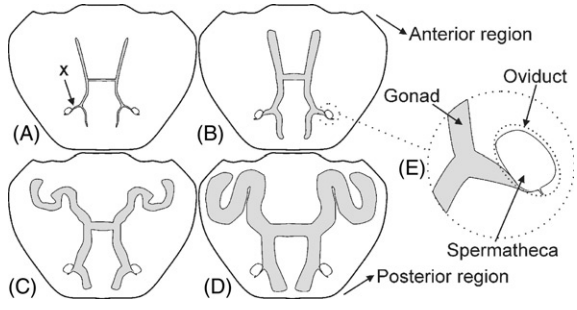

Figure 1. Schematic location of reproductive tract in the cephalothorax of *C. guanhumi*. Dorsal view, excluding the back of the carapace and other components. A, immature or resting B, maturing; C, maturing or spawning; and D, mature or spawning; E, details of oviduct position; X, region of gonad preceding the spermatheca.

condensed chromatin is adhered to the inner covering of the nucleus (Figure 3(B)), whereas the cytoplasm exhibited a weaker reaction to these stains and minimal accumulation of proteins, staining weakly by Bromophenol Blue (Figure 3(C)).

Table 1. Cell characteristics of the different oocyte types during *C. guanhumi* ovarian development.

	n	CD	ND	ND/CD (%)
OO	100	14.17 ± 2.02^{a}	7.68 ± 1.03^{a}	54.94 ± 4.55^{a}
PVO	100	51.02 ± 7.39^{b}	17.82 ± 1.48^{b}	35.41 ± 3.32^{a}
VO	50	110.93 ± 9.33^{c}	23.13 ± 2.61^{c}	21.79 ± 2.30^{b}
MO	50	237.52 ± 22.90^{d}	35.55 ± 5.06^{d}	15.61 ± 1.57^{c}

Notes: Diameter (mean ± standard deviation) of oocytes (CD) and nuclei (ND) (μm) and ratio between these means (percentage) for oogonia (OO), PVO, VO, and MO. Different letters within the same columns denote significant differences. Tukey test, for cell: $p < 0.0001$; for nucleus: $p < 0.0001$; for ND/CD: $p < 0.0001$. Cells and nuclei were analyzed separately.

Pre-vitellogenic oocytes. Diameter from 26 to 100 μm. These cells are found in the periphery of the germ zone. The cytoplasm is more developed than in the previous stage, although the basophilic nature remains (Figure 3(E)). In the nucleus, there are one or two peripheral nucleoli (generally one nucleolus).

Vitellogenic oocytes. Diameter from 79 to 160 μm. Due to the increased volume, cells are juxtaposed and their previously spherical shape becomes polyhedric (Figure 3(F)). There is a large deposit of proteins in the cytoplasm (evidenced by the intense coloration with Bromophenol Blue) that becomes a component of the yolk (Figure 3(D) Vitellogenic deposits are predominantly distributed in the peripheral region of the cytoplasm, giving it a granular appearance with

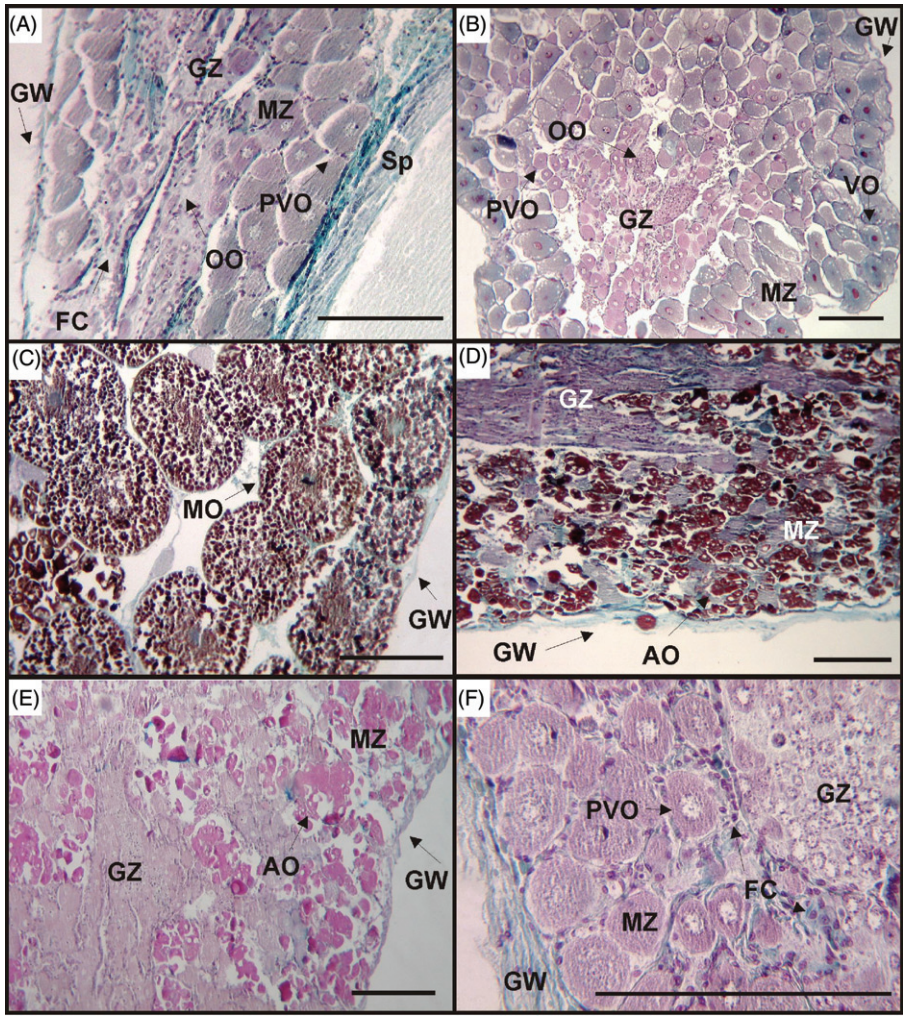

Figure 2. Photomicrographs of female gonad stages in *C. guanhumi*. A, immature; B, maturing; C, mature; D, spawning (ovigerous); E, spawning (non-ovigerous); F, resting. GZ, germ zone; MZ, maturation zone; OO, oogonia; PVO, pre-vitellogenic oocyte; VO, vitellogenic oocyte; MO, mature oocyte; FC, follicular cell; GW, gonad wall; Sp, spermatheca; AO, atretic oocyte. Staining, Gomori's Trichromic for A, B, C, and E; and Alcian/PAS Blue, for D and F. Scale bar, 200 μm.

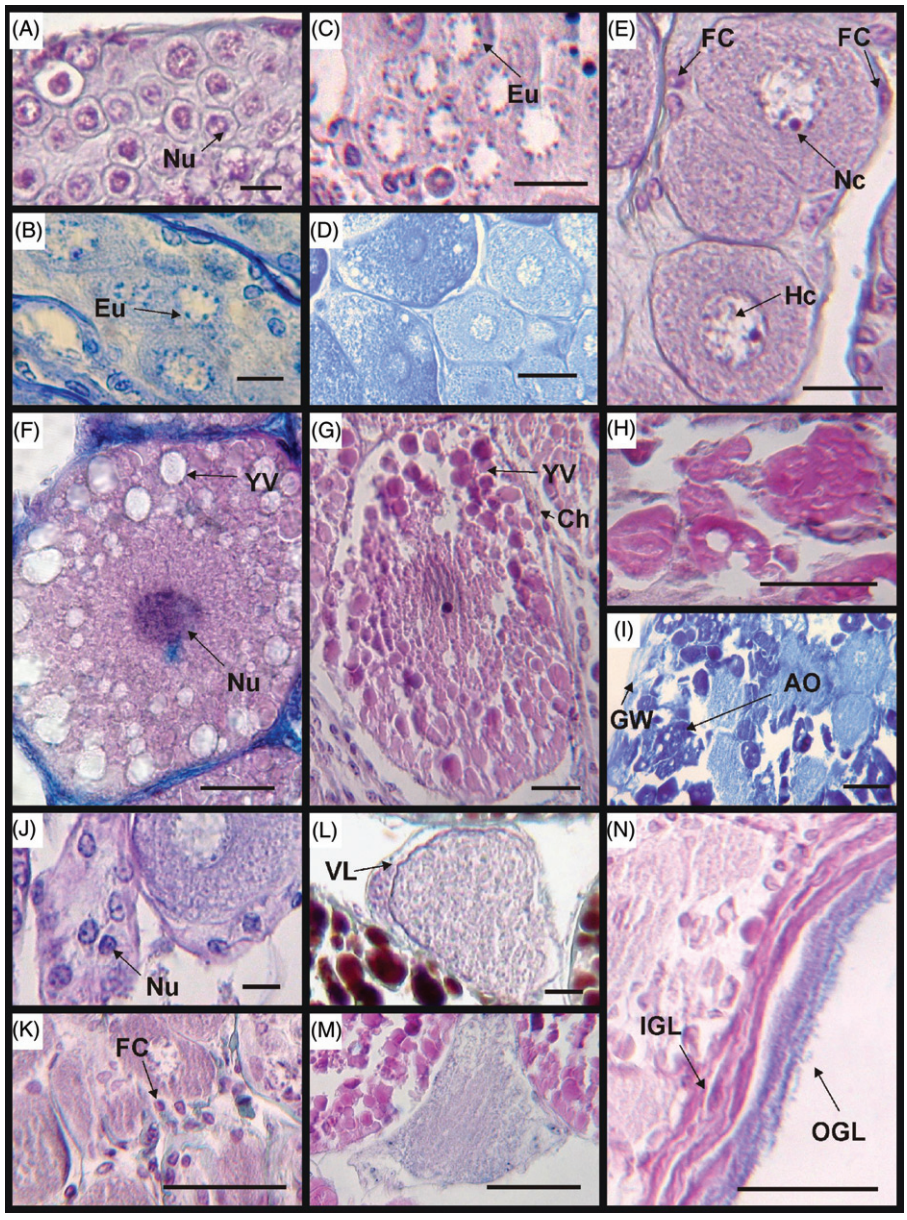

Figure 3. Photomicrographs of germ and somatic components of the ovary in *C. guanhumi*. A, oogonia in initial phase; B and C, oogonia in final phase; D, pre-vitellogenic (right side) and vitellogenic (left side) oocytes; E, pre-vitellogenic oocytes; F, vitellogenic oocytes; G, mature oocyte; H and I, atretic oocytes; J and K, follicular cells; L and M, hemal vessel; N, gonad lining. Nu, nucleus; Nc, nucleolus; Eu, euchromatin; Hc, heterochromatin; FC, follicular cell; AO, atretic oocytes; YV, yolk vesicle; Ch, Chorion; GW, Gonad wall; VL, blood vessel lining; IGL, inner gonad lining; OGL, outer gonad lining. Staining, Gomori's Trichromic (A, E, G, K, L), Bromophenol Blue (B, D, I), and Alcian/PAS Blue (C, F, H, J, M, N). Scale bar, A, B, C, D, E, J, and L = 10 μm, and F, G, H, I, K, M, and N = 50 μm.

acidophilic nature, stained lightly pink by Alcian/PAS (Figure 3(F)).

Mature oocytes. Diameter from 132 to 310 μm. Despite the large volume of the cell, in comparison to the small nucleus, it becomes barely visible. In this stage, the cell is ready to be extruded and fertilized. Large yolk vesicles are found throughout the cytoplasm, with smaller vesicles around the nucleus (Figure 3(G)). There is a thickening around the cell, stained intensely green by Gomori's Trichromic stain, called "chorion", which is an indicator of the last stage of development of germ cells.

Atretic oocytes. These are the cells that are matured but not released, thereby entering a process of re-absorption by the ovary. Atretic oocytes (AO) are acidophilic, due to the strong pink coloration, resulting

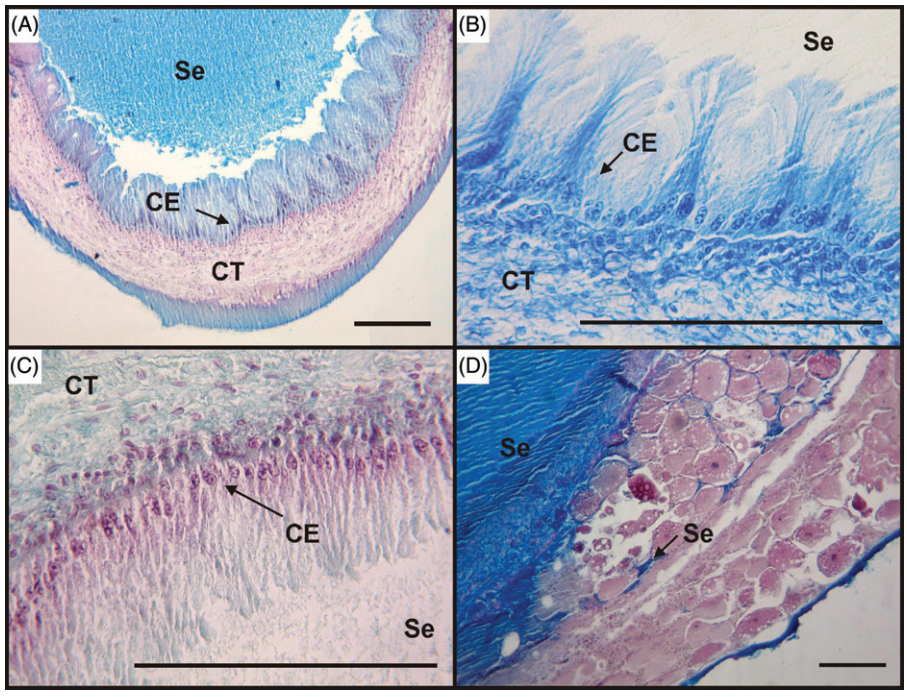

Figure 4. Photomicrographs of spermathecae sections (A, B, C) and connection region between gonad and spermatheca (D). CT, conjunctive tissue; CE, columnar epithelium; Se, secretion. Staining, Alcian/PAS Blue (A, D), Bromophenol Blue (B), and Gomori's Trichromic (C). Scale bar, 400 μm.

from staining with Alcian/PAS (Figure 3(H)) and rich in proteins, as evidenced by the staining with Bromophenol Blue (Figure 3(I)).

Follicular cells. Found free, either forming clusters or surrounding cells (Figure 3(J)), follicular cells (FCs) vary in shape from cubic and are grouped alongside the oogonia to a flattened shape when surrounding the VO or MO. FCs have little cytoplasm and the nucleus is strongly basophilic, containing peripheral nucleoli (Figure 3(K)).

Hemal vessels. Lined by a unistratified squamous epithelium, the hemal vessels (HVs) contain hemolymph and are present in the spaces between the forming units of the ovaries, where the germ cells are located (Figure 3(M)). The hemolymph has a granular appearance and reacts strongly to Gomori's Trichromic stain (Figure 3(L)), indicating a basophilic nature, whereas the lining has an even more intense reaction. The intensity of the coloration of the hemolymph increases in more advanced maturation stages and in the ovaries of ovigerous females.

Gonad wall. The ovaries are lined with a fibrous material, stained green by Gomori's Trichromic stain (Figure 2(F)). The wall is made up of an outer portion of polysaccharide acids and an inner portion of neutral polysaccharides, respectively, stained blue and magenta by the Alcian/PAS method (Figure 3(I)). When stained with Bromophenol Blue, there was a weak reaction, indicating a reduced concentration of proteins (Figure 3(I)).

Description of the spermatheca (Sp)

The lumen of this organ is lined with a columnar epithelium, with basal nuclei and an apical region of the cytoplasm which is secretory (Figure 4(A)–(C)). A large amount of secretion is accumulated filling the entire interior of the spermathecae. The secretion responded positively to the Alcian/PAS Blue stains (Figure 4(A) and (D)) and negatively to Bromophenol Blue (Figure 4(B)), indicating a non-protein nature. The outermost portion of this organ has a continuous band of Alcian/PAS-positive material (Figure 4(D)). The spermathecae do not exhibit significant changes throughout the process of gonad maturation, only an increase in volume and, as a consequence, a slight change in color to a more whitish tone. The spermathecae showed a turgid state, both in adult as well as in juvenile specimens.

Summary

The ovaries in a particular maturation stage have the same coloration and texture in the anterior, middle, and posterior lobes. The gonad is always restricted to the cephalothoracic cavity and does not reach the

pleon in any of the stages. Moreover, no differences in cell types throughout the lobes were found in the microscopic analysis, indicating that there is no difference in relation to the degree of maturation in the different parts of the same gonad (anterior or posterior). Besides, a portion termed the germ zone, the central region of the gonad in a cross-sectional cut, is present throughout the left and right lobes.

Both the macroscopic (Figure 1) and the microscopic analyses, using a light microscope (Figures 2 and 3), allowed the differentiation of the following consecutive gonad development stages (Table 2): immature, maturing, mature, spawning, and resting. Nevertheless, after the spawning stage, the ovary may either mature again or enter a resting stage, maturing again only in the next reproductive season. The immature stage is observed only in juvenile specimens that did not begin the reproductive cycle yet.

The cell composition during the different stages of gonad maturity is very typical and can be used as the main criterion for the definition of the maturity stage (Table 3). The color of the ovary may be utilized as an additional criterion for the identification of the maturity stage (Table 4), but it should be used only in conjunction with the other characteristics already described, because a similar color might be found in different stages of maturity.

Discussion

The morphology and location of the gonads in *C. guanhumi* follow the pattern already described for

Table 2. Stages of ovarian maturation based on macroscopic and microscopic characteristics in *C. guanhumi*.

Stages	Characteristics
Immature (Figures 1(A) and 2(A))	The gonads are quite small, with a uniform diameter. The spermatheca shows a reduced size, with a thin wall, and it is filled with secretion Alcian/PAS-positive. Ovaries in this stage have a translucent color, smooth texture, and thin wall. The germ zone shows the presence of oogonia, while the peripheric maturation zone holds PVO, although in lower numbers. FCs in the germ zone form nests, while in the maturation zone, around the PVO, they appear as a unistratified cubic epithelium.
Maturing (Figures 1(B) or (C) and 2(B))	Initially, the gonad lobes are expanded, with an increase in diameter. The portion that precedes the spermatheca, however, is small (Figure 1(B)), with coloration ranging from yellow to orange. The spermatheca exhibits a little increase in size and a translucent color. As development progresses, there is an increase in diameter and length of the gonad, with the anterior portion beginning to occupy the lateral region of the cephalothorax, although the region preceding the spermatheca is still small (Figure 1(C)). Ovaries in this stage have a smooth texture and color ranging from yellow to dark brown. The maturation zone, with PVO, is located more internally, while VO are situated in the more peripheral region. A large number of FCs are present in the maturation zone.
Mature (Figures 1(D) and 2(C))	The gonads occupy the entire empty space of the cephalothorax, reaching maximum ovary diameter and length. At this stage, the region preceding the spermatheca is no longer visible (Figure 4(D)). The spermatheca is more turgid and has a larger volume than in the previous stages, displaying also a more whitish color. Ovaries have a smooth texture and dark brown color, with a predominance of MO in the maturation zone and rare PVO. The germ zone remains in the central region, but it is proportionately smaller. FCs are found surrounding the MO.
Spawning (extrusion of eggs) (Figures 1(C) or (D) and 2(D) or (E))	Macroscopically, this stage might present characteristics rather similar to the maturing or mature stages. It includes females carrying eggs and those that have recently spawned them (larval hatching). Ovaries in this condition show a smooth texture and a dark brown or yellow color, containing dark brown residuals (non-eliminated oocytes). Females in this stage have AO, empty spaces, and few MO in the ovary. FCs are dispersed throughout the maturation zone in larger numbers than germ cells. In more flaccid ovaries, few AO remain, whereas the FCs and PVO begin to reorganize to occupy the empty spaces. The spermatheca is similar to the maturing stage.
Resting (Figures 1(A)and 2(F))	Macroscopically, this stage shows characteristics similar to the immature stage. After the extrusion of the eggs, the gonads reorganize and enter a resting stage. Ovaries have a translucent to yellow color and a smooth texture, similar to immature ovaries, but with a thicker gonad wall. The spermatheca remains translucent and filled with secretion Alcian/PAS-positive. FCs are found throughout all regions of the gonad, forming more than one stratum between the germ cells. There is also a greater space between the sexual cells, which is filled in by FCs and residual AO.

Table 3. Ovary cell composition from different maturation stages in *C. guanhumi*.

	Stages				
	I	II	III	IV	V
Oogonia	++	+	+	+	++
PVO	+	++	+	+	++
VO	−	++	−	−	−
MO	−	−	++	+	−
AO	−	−	−	++	+

Notes: − absent; + present; ++ abundant.
Stages: I, Immature; II, Maturing; III, Mature; IV, Spawning; V, Resting.

Table 4. Variation in gonad color (percentage) based on microscopic features, from *C. guanhumi*.

	Stages				
Gonad color/ RGB classification	I	II	III	IV	V
Translucent/RGB = 176-170-138	100.0	0.0	0.0	0.0	75.0
Yellow/RGB = 148-120-47	0.0	15.0	0.0	60.0	25.0
Orange/RGB = 162-88-13	0.0	10.0	0.0	0.0	0.0
Light brown/RGB = 85-45-28	0.0	65.0	0.0	0.0	0.0
Dark brown/RGB = 41-28-20	0.0	10.0	100.0	40.0	0.0

Note: Stages: I, Immature; II, Maturing; III, Mature; IV, Spawning; V, Resting.

other brachyurans (e.g., *Callinectes sapidus* Rathbun, 1896; *Maja brachydactyla* Balss, 1922; *Armases rubripes* Rathbun, 1897 (Johnson 1980; Rotllant et al. 2007; Santos et al. 2009; respectively)), with reports of similar characteristics for decapods (Adiyodi and Subramonian 1983).

Regarding the spermatheca, some authors have reported similar characteristics for brachyurans to those found here: the spermatheca is connected to the ovary and oviduct in its basal region, close to where it is joined to the vagina (Lautenschlager et al. 2010); with the presence of a layer of columnar epithelium (Becker et al. 2011); filling with a substance of a basophilic nature that appears to serve as support to the spermatozoa, however, without the function of nourishing them (Johnson 1980).

For *C. guanhumi*, in the present case, since no spermatozoa were found in the spermathecae, even in mature females, there may be two possibilities: Either the sample was not sufficient (138 adult females), or it is likely that they use the sperm immediately following copulation. The results found in this study resemble those described for *Libinia emarginata* Leach, 1815 (Hinsch 1986), differing, however, from those found by Taissoun (1974), who report that *C. guanhumi* females are capable of storing sperm for a long period of time.

The observation of developed ovaries while a female is brooding eggs suggests that a single individual can mature more than once per season. Corroborating this hypothesis, the occurrence of more than one spawning per season was observed for *C. guanhumi* in Florida (USA) (Gifford 1962), whereas Mendes (2004) found that ovigerous females in the laboratory became again ovigerous soon after spawning one individual.

Table 5. Maximum and minimum diameters or mean diameter (μm) of oogonia (OO), PVO, VO, and MO cited by crustaceans by different authors.

		Diameter (μm)				
Species	Family	OO	OPV	OV	OM	Authors
Panulirus japonicus	Palinuridae	10–14	13–43	23–451	465–477	Minagawa and Sano (1997)
Penaeus penicillatus	Penaeidae	17–65	70–125	100–200	125–250	Ayub and Ahmed (2002)
Penaeus merguiensis	Penaeidae	17–65	70–125	100–200	125–250	Ayub and Ahmed (2002)
Metapenaeus affinis	Penaeidae	11–60	70–112	75–175	100–175	Ayub and Ahmed (2002)
Parapenaeopsis stylifera	Penaeidae	11–60	60–112	75–150	100–175	Ayub and Ahmed (2002)
Farfatepenaeus paulinsis	Penaeidae		25–100	100–220	220–340	Peixoto et al. (2003)
Uca rapax	Ocypodidae	10–22	40–80		160–280	Castiglioni et al. (2007)
M. brachydactyla	Majidae	5–6	264 (mean)	453–673 (mean)	845 (mean)	Rotllant et al. (2007)
Cherax quadricarinatus	Parastacidae	11–14 (mean)	26–544	106–581	181–1629	Vazquez et al. (2008)
G. cruentata	Grapsidae	10 (mean)	20–80	50–340	250 a 410	Souza and Silva (2009)
Palinurus elephas	Palinuridae	14–32	40–160	135–956	865–1285	Rjeibi et al. (2010)
Scylla paramamosain	Portunidae	5–10	45–100	80–200	150–250	Islam et al. (2010)
Halicarcinus planatus	Hymenosomatidae		75–100		180–430	Diez and Lovrich (2010)
C. guanhumi	Gecarcinidae	9–26	26–100	79–160	132–310	Present study

Although the ovaries present a well-defined shape (Figure 4), their structure is very fragile and can be easily ruptured, (also related by Santos et al. 2009). In addition, once removed from the cephalothoracic cavity, the ovary tends to lose its original shape, making the measurement of its length or width very imprecise. Furthermore, in the immature and resting stages, because of the reduced size of the ovaries and to avoid their rupture, they were removed with parts of the hepatopancreas, rendering the measurement of weight and volume highly inaccurate.

Different color classifications for *C. guanhumi* have been attributed to the gonads in different stages of maturity. In this study, the scale varies from translucent, yellow, orange, light brown until dark brown. Botelho et al. (2001) described from transparent to whitish/milky, yellow/orangey, and dark brown and Silva and Oshiro (2002) ranked as transparent, butter/ivory, orangey yellow/mustard, or dark brown, nearly black. However, these authors have classified the colors without any standardization, which makes the comparison between them difficult. It is likely that the disagreement in color determination is merely due to the name of the color or its intensity, since the results of the analysis of the germ cells and their components are generally quite similar. Due to this difficulty, we recommended that a universally recognized color scale be adopted, as suggested by Peixoto et al. (2003).

The diameter of the oocytes appears to be characteristic for each species, with a broad range of values described by different authors (Table 5). All studied species, however, show an increase in diameter throughout the development process, with the families Grapsidae, Penaeidae, Portunidae, Hymenosomatidae, and Gecarcinidae with the most similar values when compared with other families. Such variation is probably related to the accumulation of yolk, which occurs in a different manner for each species or family. Although the use of different methods for the study of oocytes might result in different levels of dehydration, within a uniformly applied method, such spurious effects would tend to affect all cells in a relatively equal manner, thus reducing the risk of a relative bias between cells.

For a better understanding of the stages of germ cell development, it is important to link the somatic content of the ovaries. In this context, Souza and Silva (2009), studying *Goniopsis cruentata* Latreille, 1802, noted that few studies give these detailed descriptions. According to the author, the fundamental role the FCs play in gonad maturation is the synchronic maturation, thereby ensuring the amount of MO necessary for spawning. In addition, Chang and Shih (1995) reported that the format and size of these cells are related to the biosynthetic activity.

A number of authors do not include in their analyses of maturation stages that occur between spawning and the beginning of a new cycle, and this lack can cause problems in the estimation of size at first maturity, because resting individuals may be wrongly classified as immature. A similar problem can be seen in studies that use only macroscopic classification. From our results, we infer that *C. guanhumi* has a gonad maturation that can be divided into five different stages of maturity based on the macroscopic and microscopic characteristics of the gonads (Table 2) and on the cell composition (Table 3), in association with both changes in color (Table 4) and the volume they occupy in the cephalothorax cavity.

References

Adiyodi RG, Subramonian T. 1983. Arthropoda-Crustacea. In: Adiyodi KG, Adiyodi RG, editors. Vol. 1. Reproductive biology of invertebrates, oogenesis, ovoposition and oosorption. Toronto: John Wiley and Sons. p. 443–495.

Ayub Z, Ahmed MA. 2002. Description of the ovarian development stages of penaeid shrimps from the coast of Pakistan. Aquaculture Research. 33(10):767–776.

Becker C, Brandis D, Storch V. 2011. Morphology of the female reproductive system of European pea crabs (Crustacea, Decapoda, Brachyura, Pinnotheridae). Journal of Morphology. 272(1):12–26.

Botelho ERO, Santos MCF, Souza JRB. 2001. Aspectos populacionais do guaiamum, *Cardisoma guanhumi* Latreille, 1825, do estuário do Rio Una (Pernambuco - Brasil). Boletim Técnico e Científico CEPENE, Tamandaré. 9(1):123–146.

Burggren WW, McMahon BR. 1988. Biology of the land crabs. New York: Cambridge University Press.

Castiglioni DS, Negreiros-Fransozo ML, López-Greco LS, Silveira AF, Silveira SO. 2007. Gonad development in females of fiddler crab *Uca rapax* (Crustacea, Brachyura, Ocypodidae) using macro and microscopic techniques. Iheringia Série Zoologia. 97(4):505–510.

Chang CF, Shih TW. 1995. Reproductive cycle of ovarian development and vitellogenin profiles in the freshwater prawns, *Macrobrachium rosenbergii*. Invertebrate Reproduction and Development. 27(1):11–20.

Diez MJ, Lovrich GA. 2010. Reproductive biology of the crab *Halicarcinus planatus* (Brachyura, Hymenosomatidae) in sub-Antarctic Waters. Polar Biology. 33(3):389–401.

Gifford CA. 1962. Some observations on the general biology of the land crab *Cardisoma guanhumi* (Latreille), in South Florida. The Biological Bulletin. 123(1):207–223.

Hinsch GW. 1986. A comparison of sperm morphology, transfer and sperm mass storage between tow species of crab, *Ovallipes ocellatus* and *Libinia marginata*. International Journal of Invertebrate Reproduction. 10:79–87.

Islam MS, Kodama K, Kurokura H. 2010. Ovarian development of the mud crab *Scylla paramamosain* in a

tropical mangrove swamps, Thailand. Journal of Scientific Research. 2(2):380–389.

Johnson PT. 1980. Histology of the blue crab *Callinectes sapidus*: a model for the Decapoda. New York: Praeger.

Lautenschlager AD, Brandis D, Storch V. 2010. Morphology and function of the reproductive system of representatives of the genus *Uca*. Journal of Morphology. 271(11): 1281–1299.

Mendes PP. 1999. Estatística aplicada à aqüicultura. Bargaço: Recife-PEp. 265.

Mendes LN. 2004. Reprodução do caranguejo guaiamum, *C. guanhumi* Latreille, 1825 (Crustácea, decapoda) capturado em manguezais do estado do Ceará. Fortaleza/CE [Dissertação]. [Mestrado em Engenharia de Pesca]: Universidade Federal do Ceará. p. 43.

Minagawa M, Sano M. 1997. Oogênesis and development cycle of the spiny lobster *Panulirus japonicus* (Decapoda: Palinuridae). Marine and Freshwater Research. 48:875–887.

Pearse AGE. 1960. Histochemistry theoretical and applied. Vol. 2. London: Jet. Churchill Ltd. p. 965.

Peixoto S, Cavalli RO, D'Incao F, Milach AM, Wasielesky W. 2003. Ovarian maturation on wild *Farfatepenaeus paulinsis* in relation to histological and visual changes. Aquaculture Research. 34(14):1255–1260.

Rjeibi O, Gaamour A, Missaoui H. 2010. Kinetics of oogenesis and spawning strategy of the red spiny lobster *Palinurus elephas*. Journal of Crustacean Biology. 30:401–412.

Robinson A, Morrison J, Muehrcke P. 1995. Elements of cartography. 6th ed. New York: John Wiley and Sonsp. 688.

Rotllant G, González-Gurriarán E, Fernández L, Benhalima K, Ribes E. 2007. Ovarian maturation of the multi-spawning spider crab *M. brachydactyla* (Decapoda: Majidae) with special reference to yolk formation. Marine Biology. 152(2):383–394.

Santos CM, Lima GV, Nascimento AA, Sales A, Oshiro LMY. 2009. Histological and histochemical analysis of the gonadal development of males and females of *Armases rubripes* (Rathbun 1897) (Crustacea, Brachyura, Sesarmidae). Brazilian Journal of Biology. 69(1):161–169.

Sastry AN. 1983. Ecological aspects of reproduction. In: Watermann TH, editor. The biology crustacea. VII. Environmental adaptations. New York: Academic Press p. 179–270.

Shaw BL, Battle HI. 1957. The gross and microscopic anatomy of the digestive tract of the oyster *Crassostrea virginica* (Gmelin). Canadian Journal of Zoology. 35:325–347.

Silva R, Oshiro LMY. 2002. Aspectos reprodutivos do caranguejo Guaiamum, *Cardisoma guanhumi* Latreille, 1828 (Crustacea, Decapoda, Brachyura) da Baía de Sepetiba. RJ. Revista Brasileira de Zoologia, Curitiba/PR. 19(3):907–914.

Souza LP, Silva JRF. 2009. Morphology of the female reproductive system of the red-clawed mangrove tree crab (*Goniopsis cruentata*). Scientia Marina. 73(3):527–539.

Taissoun NE. 1974. El cangrejo de tierra *Cardisoma guanhumi* (Latreille) en Venezuela. Boletín del Centro de Investigaciones Bioloógicas. Venezuela. 10:1–41.

Tolosa EMC, Rodrigues CJ, Behemer OA, Freitas-Neto AG. 2003. Manual de técnicas histológica normal e patológica. Manole: São Paulo.

Vazquez FJ, Tropea C, López-Greco L. 2008. Development of the female reproductive system in the freshwater crayfish *Cherax quadricarinatus* (Decapoda, Parastacidae). Invertebrate Biology. 127(4):433–443.

Zar JH. 1984. Biostatistical analysis. Englewood Cliffs (NJ): Prentice Hallp. 620.